

Remarks

Claims 8-11 and 14-16 are pending in the application. Reconsideration and allowance is requested in view of the above changes and the following remarks.

Applicant notes the withdrawal of the Section 112 rejections, and the withdrawal of the Section 102(e) rejection of claims 8-11 and 14-16 over Srivastava *et al.* (US Pat. 6,048,530).

Response to Section 103 Rejection

The claims remain rejected under 35 USC 103 over Srivastava *et al.* (US Pat. 6,048,530). Reconsideration is requested.

In his previous response to this rejection, applicant submitted that Example 5 of the instant specification showed that the immune response mediated using immunogenic compositions of the invention was not based on a dose-related effect. It was further submitted that TNF-induced cells showed a 10-100 fold higher antibody titer than those immunized with constitutively expressed heat shock proteins.

Examiner was not persuaded by these arguments on the grounds that it would be both known and expected from the art that heat shock results in increased levels of heat shock protein, complexes being produced, comparative to levels present in unstressed cells, where the heat shock proteins are only expressed at constitutive levels. Accordingly, examiner concludes that there would be more immunogen present in stressed cells (by virtue of the enhanced heat shock protein production which results from the heat shocking of the cells), and that it would be this increased amount of stress protein complexes (as opposed to the enhanced immunogenicity of the stress protein complexes induced by heat shock) that causes the enhanced immunogenic effect.

In this regard, examiner further submits that as Example 5 does not include a control to take into account the possible increase in the amount of heat shock proteins which may be present as a result of stressing of the cell, nor any quantification step which would allow for the amount of (stress) protein which is present in the complexes which were administered to individuals, the effect of an enhanced dose of stress protein complexes being responsible for the observed higher immunogenicity cannot be discounted.

Applicant however maintains that there is an appreciable difference and distinction between the stress protein complexes derived from stressed cells (produced in accordance with the invention), when used to mediate an immune response, and the constitutively expressed stress protein complexes which are present in the non-stressed cells of Srivastava.

In particular, applicant maintains the position that the enhanced immunogenicity which results from the use of the stress induced heat shock protein-peptide complexes of the invention is a virtue of the complex being more immunogenic *per se*. This enhanced immunogenicity is a result of the stress induced heat shock protein forming a complex with a peptide which, in turn, mediates a greater immune response, when administered to a subject, than with a peptide which would be associated with a non-induced, constitutively expressed heat shock protein, such as those of Srivastava.

The enhanced immune response of the instant invention is *not* attributable to the stress stimulus inducing more heat shock proteins, and therefore a higher dose being provided. Hence, put crudely, the induced heat shock protein-peptide complexes of the instant invention would be more immunogenic, when administered to a subject, than the non-induced constitutively expressed heat shock protein complexes, when compared on a "pound-versus-pound" basis.

To support the applicant's position, applicant submits herewith a scientific journal document in the name of Callahan, M.K. *et al. The Journal of Biological Chemistry*, 2002, Vol 277, No 37, pp33604-33609 ("Callahan *et al.* 2002).

Callahan *et al.* 2002 shows that the heat shock protein, HSP70 has 2 closely homologous genes hsc70, which is constitutively expressed and hsp70, which is stress-inducible. Both genes would be present in cells infected by the pathogenic pathogens defined in the present invention. In particular, hsp70 would be induced by a stress induced stimulus, as disclosed in the instant application, whereas in the Srivastava document cited by the examiner, the hsc70 would be the main species which forms any stress protein complexes in the Srivastava patent, as there is no used of a stress stimulus.

Callahan *et al.* 2002 compares hsc70 to hsp70 and shows that they are significantly different, both in terms of structure and function. More importantly, and of particular relevance to the applicant's assertions provided herewith, is the finding that the formation of stress protein

complexes (heat shock protein complexes) by hsp70 is enhanced by stress. It is therefore concluded that hsp70 production is enhanced by stress and that stress protein complexes formed by hsp70 exhibit enhanced immunogenicity.

Specific passages of relevance of Callahan *et al.* 2002 can be found as follows:

Page 33604, column 2, paragraph 3, which reads “*some HSPs are stress-inducible, like Hsp70, whereas some are constitutively expressed, like Hsc70*”.

Page 33608, column 2, paragraph 2 states “*we have shown that Hsp70 is a better peptide binder than Hsc70 in every condition tested*”.

Page 33609, column 1, paragraph 3 concludes “*The observation that Hsp70 associates with immunogenic peptides quantitatively better than Hsc70 in all of the conditions tested brings a new element in trying to explain the different immunological properties of the 2 chaperones. It suggests that Hsp70 could be a chaperone designed to preferentially capture and deliver polypeptidic information to the immune system under stress*”.

Accordingly, the teachings of Callahan *et al.* 2002 make a clear distinction between an induced (Hsp70) stress protein complex, according to the present invention, and to a constitutively (non-induced) Hsc70 stress protein complex, which is consistent with that disclosed by Srivastava, as no form of stress is used to subject the cells to a stress-inducing stimulus in the Srivastava document. Further, although mention is made in Srivastava that heat shock protein production can be enhanced by a stress stimulus (see column 5, lines 35 and 36), none of the examples identify the use of such a stress. Hence, although Srivastava refers to inducible (Hsp70) stress proteins, it in fact exemplifies Hsc70 stress protein, as Srivastava was not aware of the distinction between constitutively expressed heat shock proteins and induced heat shock proteins. Accordingly, as the Srivastava document does not appreciate that a different, more immunogenic subset of stress protein complexes can result following a stress stimulus, there is no teaching, or motivation in the Srivastava document which would motivate the skilled person to modify the teaching of Srivastava to use a stress protein stimulus.

In the office action issued on this application of May 28, 2008, examiner states that “...*Applicants still have not demonstrated any differences in the TNF-stressed or heat-stressed compositions as modified*”. It is submitted that the journal article of Callahan *et al.* 2002 as submitted herewith provides evidence which supports the fact that Srivastava does not employ the use of a stress stimulus, and therefore that induced Hsp70 production does not result, and that the stress proteins complexes of Srivastava are derived from the constitutively expressed Hsc70 stress proteins, rather than the more immunogenic induced Hsp70 complexes.

Claim 8 as pending, recites the requirement for “*an induced stress protein*”. The claim therefore requires that the immunogenic determinant complex comprises an induced stress protein, *i.e.*, an Hsp70 stress protein, as opposed to a non-induced constitutively expressed Hsc70 protein, as would be provided by the invention of Srivastava, due to the absence of a stress stimulus.

Furthermore, applicant also submits that the requirement of claim 8 that the immunogenic determinant contains “*an antigenic peptide fragment obtained from a cell which has been infected with a bacterial, protozoal or parasitic fragment, which infected cell has been subjected to stress from heat or tumour necrosis factor...*” also results in a stress protein-peptide complex which exhibits a structural difference over the stress protein-peptide complexes which are provided by Srivastava – these being complexes comprising non-induced constitutively expressed proteins. In this regard, applicant refers to the further cited scientific journal paper of Callahan *et al.* (Callahan, M.K *et al.*, “Heat Shock Up-Regulates Imp2 and Imp7 and Enhances Presentation of Immunoproteasome-Dependent Epitopes” *The Journal of Immunology*. 2006. 177, page 8393-8399 (“Callahan *et al.* 2006”))

Callahan *et al.* 2006 studies the effect of heat shock on gene expression of genes involved with MHC I pathway antigen presentation. The MHC I antigen presentation pathway is the pathway in which the stress proteins of the invention are involved. Specifically, Callahan *et al.* 2006 shows that “*two inducible subunits of the proteasome, Imp2 and Imp7, are transcriptionally upregulated by heat shock*” (see abstract). The proteasome has an important role in antigen presentation, as it degrades protein in the cytosol prior to the resulting protein

fragments associating with stress proteins and forming stress protein complexes (heat shock protein-antigenic peptide fragment complexes).

The identification by Callahan *et al.* 2006 that a stress, such a heat shock, causes up-regulation of Imp2 and Imp7 expression, and that “*This upregulation has an immunological consequence in that heat-shocked cells, such as IFN- γ -treated cells, show enhanced presentation of immunoproteasome-dependent MHC I epitopes*” is significant to the instant invention, as it indirectly implies that the immunogenic complexes provided by the present invention must be immunologically superior in mediating an immune response, because the MHC binds peptides provided by the heat shock proteins. Hence, in order for the MHC to present enhanced immunogenic epitopes, the stress protein-peptide complexes which are trafficking these “immunoproteasome-dependent MHC I epitopes” to the MHC must also be structurally different, otherwise no enhancement in immunogenicity would be identified.

This effect is summarized in Callahan *et al.* 2006, which states at page 8398, column 2 that “...*a study of the relationship between the level of immunoproteasome induction and presentation of an immunoproteasome-dependent epitope showed that small changes in immunoproteasome expression cause significant changes in Ag processing. Thus, even the lower levels of induction of immunoproteasome subunits by heat shock have an effect on changing Ag presentation patterns, as indeed was shown in the present studies*”.

The teachings of the instant specification further distinguish the induced stress protein complexes of the invention from non-induced constitutive stress protein complexes of Srivastava in a number of indirect ways. Firstly, Example 5 which relates to the immunization of subjects using the stress proteins prepared in accordance with the invention refers consistently to constitutive and induced stress proteins. For example, at page 17 of the instant specification which reads “*Infected cells were used to prepare lysates for antibody titre assays, or cultured overnight in the presence or absence of 1ug/ml TNF- α at 37°C for the isolation of constitutive or TNF-induced SPs*”. Further, page 17, lines 29 and 30 read “*Constitutive and TNF-induced SPs were prepared from the cleared lysates ... as described in Example 1*”. If the applicant had not known that there was a distinct and appreciable difference between the stress protein complexes which were derived from stress induced cells and those of non-induced cells, then he would have

not have recognized the advantage conferred by, and superior properties of, the induced stress protein complexes of the instant invention for use as immunogenic determinants.

Referring to the teachings of Example 1 of the instant application, the inventor discusses the concentration of induced stress protein complexes which is required to produce a vaccine composition according to the invention. The discussion states that *"The SP complexes may be used at any suitable concentration to provide the immunogenic determinant in the vaccine composition. We prefer that the amount of induced SP complex that is administered is in the range of 10-600 ug, preferably 10-100 ug, most preferably 25 ug per kg of animal body weight"* (page 14, lines 3-6).

The inventor has, based on his knowledge of the enhanced immunogenicity of the stress protein complexes of the present invention, provided specific guidance to the user as to what concentration of induced stress protein complexes is needed to obtain the advantages of the invention – *i.e.*, the inventor has appreciated that it is the nature of the stress protein complexes, rather than amount or dosage effect of the stress protein complexes, which mediates the observed enhanced immunogenic effect.

A further example of the inventor's identification and appreciation of the fact that the induced stress protein complexes of the invention are more immunogenic, *not because there is a greater amount of stress protein following a stress stimulus, but because the induced stress proteins are intrinsically different*, is taught by the instant specification at page 5, lines 21 to 31 which states that *"It is surprising that the treatment of cells infected with an intra-cellular pathogen with heat or tumour necrosis factor produces SPs which are more immunogenic than SPs derived from non-induced cells or cells which have been stressed by other stimuli. A notable aspect of immunity elicited by these induced SPs is the long-term memory compared to that induced by immunisation by other SP subsets. The best memory responses for bacterial pathogens are seen with heat induced stress proteins and for protozoan and parasitic pathogens with tumour necrosis factor"*.

This is further supported by the passage in the instant specification at page 7, lines 17 to 21 stating that *"... it is thought that the treatment of the cells infected by intracellular pathogen operates either to induce specifically those HSPs most able to interact with antigenic peptides*

from the pathogen, or to induce those HSPs which are most easily phagocytosed by APCs, or both”.

Hence, these passages again illustrate the fact that the inventor fully appreciated that the stress protein complexes of the present invention confer an enhanced immunogenic response when administered to a subject by virtue of the unique structure of the stress protein complex, rather than merely because a greater amount of stress protein was provided in stressed cells.

In summary, applicant submits that the induced stress protein complexes are *structurally distinct* and *more immunogenic* than heat shock protein-peptide complexes disclosed by Srivastava. Simply put, there is an intrinsic difference in the complexes of the present invention over those of Srivastava, as shown both by the examples of the instant application and further by the documents cited herewith.

Reconsideration and withdrawal of the Section 103 rejection is respectfully submitted.

Other Applications of Applicant Directed to Related Subject Matter

The application was previously rejected for obviousness-type double patenting over certain claims of application No. 10/049,704. The rejection was withdrawn based upon applicant's arguments. To complete the record of the present application, the references of record from the '704 application, not already of record in the present application, are submitted herewith:

Citations from Application No. 10/049,704

US 7041465 Hultgren *et al.*
US 6962791 Hultgren *et al.*
US 6913750 Hultgren *et al.*
US 6872542 Hultgren *et al.*
US 6576244 Weltzin *et al.*
US 6500434 Langermann *et al.*
US 6455503 Srivastava
US 6447781 Srivastava
US 6410028 Srivastava
US 6368599 Langermann *et al.*
US 6248330 Labigne *et al.*
US 5747332 Wallen *et al.*
US 5961979 Srivastava

US-5981706 Wallen *et al.*

US 5843460 Labigne *et al.*

US 5561221 Yoshida *et al.*

US 5049646 Srivastava

US-2005/0232946 Calaco

US 2004/0052812 Hoe *et al.*

US 2004/0047879 Tian *et al.*

US 2004/0052812 Hoe *et al.*

US 2004/0022796 Srivastava

US 2003/0216315 Nicchitta *et al.*

US-2003/0082242 Lee *et al.*

US 2003/0211102 Tiwari

US 2003/0165516 Srivastava

US 2003/0099665 Langermann *et al.*

WO 90/02564

WO 96/40928

WO 00/10597

WO 01/13944

WO 01/63278

Bae, J. *et al.*, *Veterinary Microbiology*, 88(2):189-202, August 25, 2002

Bowie, *et al.*, *Science*, 247:1306-1310, 1990

Del Giudice, G *et al.*, *J. Immunol.*, 150(5):2025-2032, March 1, 1993

Deepe, G. *et al.*, *Infection and Immunity*, 70(7):3759-3767, July 2002

Ding, Y. *et al.*, *Biochemistry*, 34:14918-14931, 1995

Eschweiler, B *et al.*, *Inter. J. Med. Microbiology. Viol. Parasitol. Infect. Dis.* (Germany), 280(1-2):73-85, Sept. 1993

Evans, D. *et al.*, *Infection and Immunity*, 60(5):2125-2127, May 1992

Fayet, O *et al.*, *J. Bacteriol.*, 171(3):1379-1385, 1989

Ferrero *et al.*, *Proc. Natl. Acad. Sci USA*, 92(14):6499-6503, 1995

Greenspan, *et al.*, *Nature Biotechnology*, 17(10):936-7, October 1999

Hechard, C. *et al.*, *J. Med. Microbiol.*, 53(pt 9):861-868, Sept 2004

Lamiet, A. *et al.*, *The EMBO Journal*, 9(7):2315-2319, 1990

Leclercq, S *et al.*, *J. Med. Microbiol.*, 51(1):20-26, Jan 2002

Motohashi, K. *et al.*, *Proc. Natl. Acad. Sci. USA*, 96:7184-7189, June 1999

Narberhaus, *Molecular Microbiology* 31(1):1-8, 1999

Noll, A *et al.*, *Infection and Immunity*, 64(8):2955-2961, 1996

Phipps, B. *et al.*, *The EMBO Journal*, 10(7):1711-1722, 1991

Rambukkana, A. *et al.*, *Infection and Immunity*, 60(11):4517-4527, 1992

Sparrer, H *et al.*, *Proc. Natl. Acad. Sci. USA*, 94:1096-1100, 1997

Szabo, A. *et al.*, *Proc. Natl. Acad. Sci. USA*, 91:10345-10349, 1994

Tomioka, H., *Current Pharmaceutical Design*, 10(26):3297-3312, 2004
Turner, O. *et al.*, *Infection and Immunity*, 68(6):3674-3679, June 2000
Wawrzynow, A. *et al.*, *The EMBO Journal*, 14(9):1867-1877, 1995
Wilson *et al.*, *Journal of Immunological Methods* 234:137-147, 2000
Yokota, K. *et al.*, *Microbiol. Immunol.* Vol 38(5):403-405, 1994.
Zeilstra-Ryalls, J. *et al.*, *Annu. Rev. Microbiol.*, 45:301-325, 1991
Zugel *et al.*, *Clinical Microbiology Review*, 12(1):19-39, Jan. 1999

Applicant gives notice that his application Ser. No. 10/204,929, now abandoned, was rejected for obviousness-type double patenting over the present application 10/049,702 in an office action dated Feb. 21, 2006. The rejection was withdrawn when the allegedly conflicting claims were cancelled in the present application. Application Ser. No. 10/204,929 was subsequently abandoned.

To complete the record of the present application, the references of record from the '929 application, not already of record in the present application, are submitted herewith:

Citations from Application No. 10/204,929

WO 97/10002

Lodish *et al.*, *Molecular Cell Biol.*, 3rd ed., *Scientific American Books*, NY, 1995, p. 59
Jinal *et al.*, *Trends Microbiol.*, 2:89-91 (1994)
Stewart *et al.*, *Curr. Opin. Immunol.*, 15:506-510 (2004)
Yokota *et al.*, *Microbiol Immunol.* 38:403-405 (1994)
Buchmeirer *et al.*, *Science*, 248:730-732 (1990)
Fernandex *et al.*, *Infect. Immun.* 64:1968-1976 (1996)
Murray *et al.*, *Medical Microbiol.* 4th ed., Mosby 2002, pp. 429-434.
Sedger *et al.*, *J. Virol.* 68:4685-4689 (1994).

Applicant also gives notice of his application 10/363,454 (US-2005-0232946), directed to related subject matter. To complete the record of the present application, the references of record from the '454 application, not already of record in the present application, are submitted herewith:

Citations from Application No. 10/363,354

US 5961979 Srivastava

WO 00/10597

WO 01/13944

WO 01/63278

Bowie, *et al.*, *Science*, 247:1306-1310, 1990

Greenspan, *et al.*, *Nature Biotechnology*, 17(10):936-7, October 1999

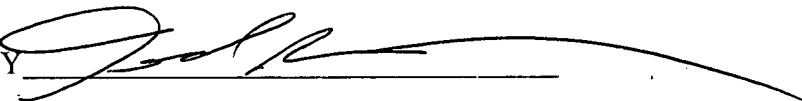
Narberhaus, *Molecular Microbiology* 31(1):1-8, 1999

Conclusion

The claims of the application are believed in condition for allowance. An early action toward that end is earnest solicited.

Respectfully submitted

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